LOW MOLECULAR NITROGEN COMPOUNDS OF MARINE ALGAE*

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Many studies devoted to the isolation of the constitutions of plants and to the determination of the structures of natural products have been conducted from the latter part of the last century. Since Tswett succeeded in the separation of leaf pigments using column chromatographic techniques at the beginning of this century, chromatographic methods prevailing at present such as paper chromatography, ion-exchange chromatography and thin-layer chromatography have been developed and established. These methods have come to be among the most potent tools the biological chemist possesses. They allow the components of a complex mixture of natural products to be resolved from each other with speed and precision, and thus can be used to detect promptly unknown substances.

With the advance of these chromatographic procedures which facilitated the discovery of additional plant products, other new physicochemical methods have been developed and applied to the structure determination. The following four techniques are of outstanding importance: infrared and ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy and mass spectrometry. By using these techniques, the newly developed physicochemical methods have become possible to determine the structures of natural products automatically.

Amino acids

Formerly it was known that twenty kinds of amino acid concerning with the synthesis of protein are produced in living organisms. However, nearly 150 amino acids have been isolated and identified chemically from organisms to date. Some of the new amino acids thus discovered and identified have been isolated and identified from marine algae. Table 1 presents a chronological list of amino- and imino-acids recently isolated from marine algae, including details of their structure, sources, and specific rotations.

As shown in Table 1, at first new derivatives of proline have been isolated from *Digenea simplex* by Murakami *et al.*[1] These amino acids are L-α-kainic acid, and its isomer, L-α-allokainic acid, and the configuration has been identified as 3-carboxymethyl-4-isopropenylproline. These substances have been synthesized.

* This article is based upon the content of a thesis delivered by the author at the symposium on the biochemistry of marine algae sponsored by the Japanese Society of Scientific Fisheries, Tokyo, in April 1968.

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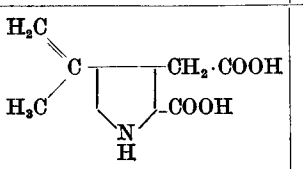
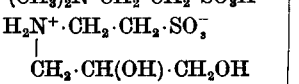
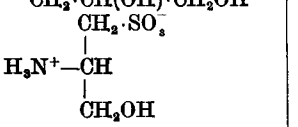
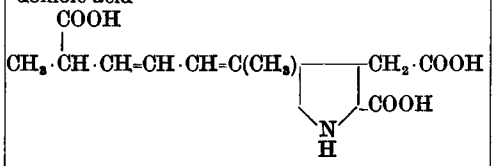
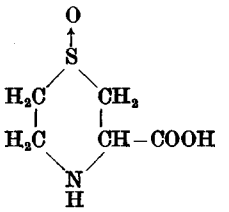
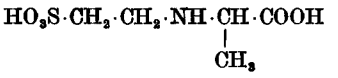
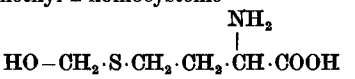
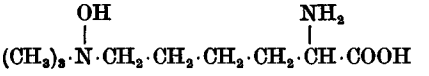
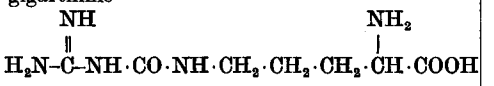
Table 1. Recently isolated amino- and imino-acids from marine algae.

Year	Investigator	Amino- and imino-acids	$[\alpha]_D$	Source
1953	Murakami, Takemoto and Shimizu	L-α-kainic acid 	−15	*Digenea simplex*
		L-α-allokainic acid	+8	
1955	Lindberg	N-methyltaurine $CH_3 \cdot NH \cdot CH_2 \cdot CH_2 \cdot SO_3H$ N-dimethyltaurine $(CH_3)_2N \cdot CH_2 \cdot CH_2 \cdot SO_3H$		*Gelidium* sp.
1956	Wickberg	D-glyceryltaurine 	−21	*Gigartina leptorhynchos*
1957	Wickberg	D-cysteinolic acid 	+7	*Polysiphonia fastigiata*
1959	Daigo	domoic acid 	−109.6	*Chondria armata*
1960	Kuriyama, Takagi and Murata	chondrine (yunaine) 	+20.9	*Chondria crassicaulis*
1961	Kuriyama	rhodoic acid 	−1.15 (N-NaOH)	*Chondrus ocellatus* *Chondrus yendoi* *Neodilsea yendoana*
1964	Takagi and Okumura	S-hydroxymethyl-L-homocysteine 	−73	*Chondrus ocellatus*
1964	Takemoto, Daigo and Takagi	laminine 	+10.8	*Laminaria angustata*
1965	Ito and Hashimoto	gongrine 		*Gymnogongrus flabelliformis*
1966	Ito and Hashimoto	gigartinine 	+7.5	*Gymnogongrus flabelliformis*

Later, N-methyltaurine and N-dimethyltaurine have been isolated from *Gelidium* sp. by Lindberg[2].

D-Glyceryltaurine[3] occurs as an important constituent of *Gigartina leptorhynchos*. This amino acid is one of the rare naturally occurring D-amino acids, and was synthesized by reductive coupling of isopropylidene-D-glyceric aldehyde with taurine. As for the distribution of this amino acid, Ito[4] has discovered that it is contained in a high proportion of 0.2 per cent in *Gymnogongrus flabelliformis* and isolated at crystalline state.

D-Cysteinolic acid has been isolated from *Polysiphonia fastigiata* and identified as D-amino acid by Wickberg[5]. Recently, Ito[6] has isolated D-cysteinolic acid in a high percentage from *Ulva pertusa* and *Enteromorpha linza*, and more or less in a lower percentage in *Sargassum serratifolium*, *S. thunbergii*, and *Hijikia fusiforme*. Takagi *et al.*[7] have detected the content of D-cysteinolic acid in *Ulva pertusa* and *Enteromorpha linza*.

3-Carboxymethyl-4-(2-carboxy-1-methylhexa-1,3-dienyl) proline, another derivative of proline has been isolated from *Chondria armata* by Daigo[8] and was named domoic acid. Domoic acid showed a marked anthelmintic effect almost equal to that of kainic acid. After that, Takemoto *et al.*[9] have modified the configuration of domoic acid as 3-carboxymethyl-4-(5-carboxy-1-methylhexa-1,3-dienyl) proline on the basis of the investigations of the nuclear magnetic resonance spectrum of trimethyl domoate and tetrahydrodomoic acid.

A new amino acid chondrine, which contains a thiazane ring, has been isolated from *Chondria crassicaulis* by Kuriyama *et al.*[10]. This amino acid is identical with yunaine which was reported a little later in the same year by Takemoto[11] to have been obtained from the same alga. Its configuration has been identified as L-1,4-thiazane-3-carboxylic acid-S-oxide configuration. Chondrine occurs in large quantities in *Chondria crassicaulis*[7], and shows a wide distribution among algae occurring in *Ulva pertusa*[7], *Enteromorpha linza*[7], *Undaria pinnatifida*[7], *Laminaria japonica*[12], *Desmarestia ligulata*[13], *Chondrus ocellatus*[14], *Neodilsea yendoana*[7], and *Laurencia nipponica*[7]. However, the physiological function of this amino acid is unknown yet.

Rhodoic acid has been isolated from *Chondrus ocellatus* by Kuriyama[15]. This name originates from the general occurrence of this acid in various kinds of Rhodophyceae such as *Chondrus ocellatus*, *Chondrus yendoi*, and *Neodilsea yendoana*. Its configuration has been assigned to N-1-carboxyethyltaurine configuration. This amino acid was synthesized with α-brompropionic acid and taurine.

S-Hydroxymethyl-L-homocysteine, a new derivative of methionine has been isolated from *Chondrus ocellatus* by Takagi *et al.*[16,17] This amino acid is hexagonal flat in crystal shape. It occurs only in some species of red algae, either in large

quantities as much as up to 0.5 per cent of dry weight in *Chondrus ocellatus*[14], or in small quantities in *Neodilsea yendoana*[7] and *Laurencia nipponica*[7].

Laminine has been isolated as a hypotensive constituent from *Laminaria angustata* by Takemoto *et al.*[18] The configuration of this amino acid was regarded as trimethyl-(5-amino-5-carboxypentyl)-ammonium hydroxide which is a quarternary ammonium base methylated to the ε-nitrogen atom of lysine. This was synthesized by methylation of copper complex of L-lysine with dimethylsulfate. This amino acid shows a wide occurrence in varieties of Laminariaceous algae such as *Laminaria* spp.[19], *Kjellmaniella crassifolia*[19], *Costaria costata*[19], *Eisenia bicyclis*[19], *Ecklonia cava*[19], *Undaria pinnatifida* [19], and *U. undarioides*[19], but in extremely small quantities.

Gongrine has been isolated only from *Gymnogongrus flabelliformis* by Ito and Hashimoto[20] as γ-guanylureido-butyric acid. Gigartinine has also been isolated from the same alga by the same researchers[21] as L-α-amino-δ-guanylureido-valeric acid. These two amino acids are known to have a guanylurea radical and contain more than 50 per cent of the extractive nitrogen of the alga. These amino acids presumably take part in nitrogen metabolism together with arginine and citrulline.

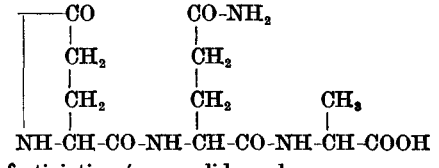
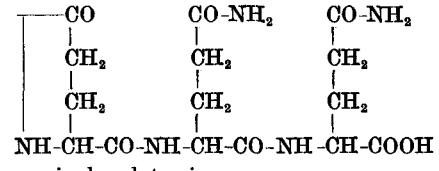
As to most of these new amino acids, very little information is available at present concerning their physiological function, the mechanism of biosynthesis, and the occurrence of the enzymes catalized in their biosynthesis. Further investigations are still to be made to clarify these natures and to discover other unknown amino acids expected to exist.

Moreover, informations about free amino acid compositions of some marine algal species have occasionally been useful for comparative biochemistry and marine algal taxonomy. With regard to the free amino acids of marine algae, their occurrence in green, brown or red algae is not determined by any rule but depends only upon the specific characters of individual marine algae. In *Ulva pertusa* and *Enteromorpha linza*[7] D-cysteinolic acid is rich while taurine is extremely poorer than in red algae. Proline is comparatively dominant, but citrulline, ornithine, and methionine appear to be absolutely absent. In *Undaria pinnatifida*,[7] higher percentages of alanine, glycine, and proline are contained than in green and red algae, but citrulline and ornithine have not been discovered yet. *Laminaria japonica*[12] has been shown to have large quantities of glutamic acid, aspartic acid, and proline, but to lack either citrulline or ornithine. Chondrine occurs in *Chondria crassicaulis*[7], taurine in *Neodilsea yendoana*[7], glutamic acid and valine in *Laurencia nipponica*[7], S-hydroxymethyl-L-homocysteine, glutamic acid, citrulline, and taurine in *Chondrus ocellatus*[14], gigartinine, gongrine, citrulline, D-glyceryltaurine, glutamic acid, and taurine in *Gymnogongrus flabelliformis*[22,23] respectively in large quantities.

Peptides

The general procedure for the isolation of the peptides occurring in marine algae was as follows. The air-dried material was digested three times in water over a boiling water bath, each for about 30 min. The resulting extract was poured off and the residual plant material was freed from adherent liquid in a tincture press. The combined extracts were then precipitated with basic lead acetate, and the filtrate, after freed from Pb with H_2SO_4, was treated with $Ba(OH)_2$ to remove SO_4 and was concentrated under reduced pressure; then the solution was precipitated by mercuric acetate, and the Hg salt, after washing, was decomposed with H_2S, filtered from HgS and evaporated to dryness in a vacuum. The peptides thus isolated from marine algae are shown chronologically in Table 2.

Table 2. Some peptides isolated from marine algae.

Year	Investigator	Peptides	Source
1931	Haas and Hill	octaglutamic acid	*Pelvetia canaliculata*
1933	Haas and Hill	pentaaspartic acid	*Corallina officinalis*
1939	Ohira	eisenine (L-pyroglutamyl-L-glutaminyl-L-alanine) 	*Eisenia bicyclis*
1949	Dekker, Stone and Fruton	fastigiatine (L-pyrrolidonoyl-α-L-glutaminyl-L-glutamine) 	*Pelvetia fastigiata*
1959	Makisumi	L-arginyl-L-glutamine	*Cladophora* sp.
1967	Konagaya	eisenine (L-glutaminyl-L-glutaminyl-L-alanine)	*Ecklonia cava*

Formerly, Haas and Hill[24,25] have isolated octaglutamic acid and pentaaspartic acid from *Pelvetia canaliculata* and *Corallina officinalis* respectively.

Eisenine has been isolated from *Eisenia bicyclis* by Ohira[26,27], and its configuration was identified as L-pyroglutamyl-L-glutaminyl-L-alanine.

Thereafter Dekker *et al.*[28] have isolated L-pyrrolidonoyl-α-L-glutaminyl-L-glutamine from *Pelvetia fastigiata* and named it fastigiatine.

Channing and Young[29] have succeeded easily in isolating the peptide occurring in *Pelvetia canaliculata* as N-dinitrophenyl derivative.

L-Arginyl-L-glutamine has been isolated by Makisumi[30] from *Cladophora* sp., a member of freshwater algae.

Discussions on the configuration of eisenine have been made for a long time whether it belongs to the ring or chain structure. Recently Konagaya[31,32] has attempted to reveal the existence of the glutaminyl peptide in an aqueous extract of *Ecklonia cava* and succeeded in obtaining the peptide, as N-dinitrophenyl derivative, and decided that L-glutaminyl-L-glutaminyl-L-alanine had the chain structure. Eisenine is quite labile to slight acidification or heating of solutions and easily cyclize to pyroglutamyl peptide.

Little information is available concerning the mechanism of the biosynthesis of peptides, Haas[33] has investigated the seasonal variations of the peptide in *Griffithsia flosculosa*, and has shown that the deficiency of illumination takes part in the formation of the peptide because of the biuret reaction of the alga appearing to be either completely negative or extremely feeble in the summer season. Moreover, as the algae were covered with a calcareous sheath in *Corallina officinalis* and *C. squamata*, the light penetration was impeded, and consequently the abnormal metabolism was caused by the photosynthesis acts on the formation of the peptides in both algae.

As the possibilities of the existence of new peptides other than the above-mentioned in marine algae seem extremely high, biosynthetic mechanism and physiological function of the peptides should be investigated in near future in an expectation of discovery of new peptides.

Nucleotides of marine algae are excluded from the present review because no remarkable development along this line has been reported up to date.

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